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Coliphages as Indicators of Enteroviruses

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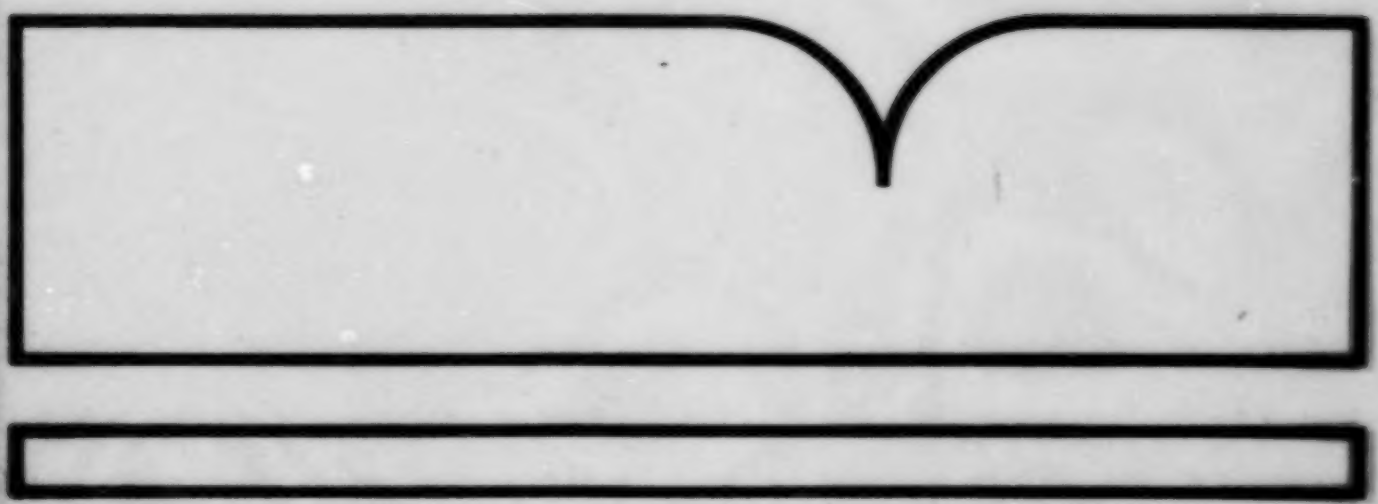

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16. ABSTRACT

Coliphages were monitored in conjunction with indicator bacteria and enteric viruses in a drinking water plant modified to reduce trihalomethane (THM) production. Coliphages could be detected in the source water by direct inoculation and sufficient coliphages were detected in enteric virus concentrates to permit following coliphage levels through different water treatment processes. Recovery efficiency by different filter types ranged from 1% to 53%. Coliphages, detected on E. coli A-19, and enteric viruses were removed at similar rates by the different treatment processes. Coliphages were not detected in finished water.

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Coliphages as Indicators of Enteroviruses

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Coliphages were monitored in conjunction with indicator bacteria and enteroviruses in a drinking-water plant modified to reduce trihalomethane production. Coliphages could be detected in the source water by direct inoculation, and sufficient coliphages were detected in enterovirus concentrates to permit following the coliphage levels through different water treatment processes. The recovery efficiency by different filter types ranged from 1 to 53%. Statistical analysis of the data indicated that enterovirus isolates were better correlated with coliphages than with total coliforms, fecal coliforms, fecal streptococci, or standard plate count organisms. Coliphages were not detected in finished water.

Coliphages have been suggested as indicators of water pollution and as possible models for enteroviruses during treatment of drinking water and wastewater (3, 6, 7, 11). Reports suggest that coliphages and enteroviruses are removed at comparable rates during treatment processes, that certain coliphages are at least as resistant to environmental stresses and to chlorination as enteroviruses, and that coliphages exhibit a seasonal variation similar to that of enteroviruses (1, 7, 14). Also, both coliphages and enteroviruses have been found in chlorinated drinking water, sometimes in the same samples (1a, 5, 7, 9, 13). However, others have reported that coliphage concentrations may increase under suitable environmental conditions, that coliphages may be present in the absence of detectable viruses, and, vice versa, that enteroviruses may be present in the absence of coliphages (1a, 5, 17).

Several criteria have been proposed for an ideal microbial indicator of pathogens (2). (i) An indicator should always be present when pathogens are present and absent when pathogens are absent. (ii) The persistence and growth characteristics of both indicator and pathogen should be similar. (iii) The pathogens and indicator should occur in a constant ratio so that counts of the indicator give a good estimate of the numbers of pathogens present. (iv) Preferably, the indicator should be present in the source of pollution at levels far in excess of the pathogen concentration. (v) The indicator should be resistant to the environment and disinfectants at the same rate as pathogens. (vi) The indicator should be nonpathogenic and easily quantifiable. (vii) The test for the indicator should be applicable to all types of water. (viii) The test should detect only the indicator organism and should not give false-positive reactions. The study reported here, carried out to determine how well coliphages meet certain of these criteria, was part of a larger study that determined enterovirus and indicator bacteria concentrations in a water treatment system modified to reduce trihalomethane production. Those results have been reported (16).

Coliphage concentrations were determined at several points during drinking-water treatment at a plant located in southern Michigan. These included the source water and treated water after flocculation-sedimentation, sand filtration, and chlorination. The conditions of the treatment were previously described (16). Coliphage concentrations in the source water were determined both by direct plaquing of grab samples and by plaquing samples concentrated by a filter adsorption-elution technique used to concentrate enteroviruses (16). Treated water samples were examined for coliphages only after concentration. The source water sample and concentrations were held at 4°C until assayed. The volumes of water concentrated were variable, depending on the sampling point and the quality of water, but were always greater than 114 liters. The volumes of sand-filtered and chlorinated waters were usually 1,900 liters, the maximum volume concentrated. Three filter types were used to concentrate entero- and bacterial viruses: an electronegative Balston filter (grade C; Balston, Inc., Lexington, Mass.) and two electropositive filters (1MDS and TSM; AMF Corp., Cuno Division, Meriden, Conn.). Viruses were eluted with 1,100 ml of 1% beef extract (pH 9.5), and a small volume was directly examined for coliphage PFU or diluted in nutrient broth before analysis.

The host bacteria used for the coliphage plaque assay were *Escherichia coli* A-19 (Hfr strain, from R. L. Ward) and *E. coli* C (ATCC 13706). An evaluation of several host bacteria, including *E. coli* B (ATCC 11303) and *E. coli* C-3000 (15597), indicated *E. coli* A-19 recovered more coliphages than the other Hfr strain, but *E. coli* C consistently yielded the highest recovery of all strains tested (data not shown). The coliphage plaque assay followed the procedures of Ward and Mahler (18). Because very low coliphage numbers were anticipated in the finished water samples, the enrichment step was used. For this, 90 ml of the finished water concentrate was split equally and mixed with 45 ml of 2× growth medium. One sample was inoculated with 1.0 ml of *E. coli* A-19, and the other was inoculated with 1.0 ml of *E. coli* C. Both were placed in a 37°C shaker-incubator overnight and then assayed for coliphage.

Table 1 summarizes the monthly coliphage recoveries obtained by direct inoculation of source water. Coliphages were detected in 31 of 39 samples assayed on *E. coli* A-19 and in 37 of 39 samples assayed on *E. coli* C. Average recoveries on *E. coli* C were 2.9 times greater than they were on *E. coli* A-19. During the fall-winter period (September 1981 to February 1982), coliphage recoveries on *E. coli* A-19 and C averaged 2 and 3.5 times, respectively, those of the spring-summer period (June to August 1981 and March to May 1982). When enterovirus concentrations from identical periods were compared, the fall-winter concentrations were four times those of the spring-summer period (16). These data confirm that coliphage and enterovirus concentrations in water fluctuate throughout the year. They also indicate that this seasonal fluctuation follows a similar pattern for the two virus groups, a phenomenon previously reported (14).

When filter concentrate coliphage concentrations were compared with the values obtained by direct inoculation of source water samples, the average recovery was 11% with

TABLE 1. Coliphage counts found by direct inoculation of source water

Date	Coliphage count[a] on:	
	E. coli A-19	*E. coli* C
6/81	1,500 (200–3,000)	1,300 (200–2,400)
7/81	1,060 (400–2,000)	4,460 (500–8,000)
8/81	<200 (<200–<200)	<465 (<200–1,000)
9/81	1,400 (600–2,200)	6,200 (4,800–7,600)
10/81	960 (400–1,600)	3,840 (1,000–8,000)
11/81	900 (200–1,600)	2,200 (1,200–3,200)
12/81	1,265 (800–2,000)	4,135 (1,800–8,200)
1/82	1,035 (400–1,670)	2,000 (1,000–3,000)
2/82	2,200 (1,000–3,400)	6,900 (4,400–9,400)
3/82	<1,160 (<200–3,200)	2,695 (1,070–6,400)
4/82	<400 (<200–800)	1,200 (400–2,000)
5/82	<200 (<200–<200)	200 (200–200)

[a] Values presented are the means of two to five samples during the months specified, and the values in parentheses are the ranges of concentrations obtained. Based on 39 samples, the average coliphage counts (PFU/liter) were 1,025 on strain A-19 and 2,965 on strain C.

the Balston filter, 20% with the 1MDS filter, and 21% with the TSM filter, the latter being the least variable (Table 2). Low rates of coliphage recovery can partially be explained because 1% beef extract (pH 9.5), a good virus eluant, was found to be a relatively poor coliphage eluant. Subsequent to the development of this study, a report was published indicating that 4% beef extract in 0.5 M NaCl (pH 9.5) was a better coliphage eluant (15). Another important factor that probably influenced coliphage recovery was the concentration of large sample volumes, a condition shown to decrease coliphage recovery (8, 15). Another factor that undoubtedly decreased recovery with the Balston filter was the low pH (3.5) used to concentrate the viruses. Others have shown that acid concentration affects coliphage survival and is selective for acid-tolerant coliphages (10, 12). A factor that influences coliphage recovery, especially with the electropositive filters, is the single-pass elution. Data obtained in this laboratory with poliovirus 1 clearly indicated that a single elution step permitted recovery of only one-third of the adsorbed or elutable virus and that reelution is necessary to improve virus recovery (unpublished data). No seeded coliphage studies were done to determine whether coliphages behave similarly, but other investigators have reported that a double elution improves coliphage recovery (15). Regardless of the poor concentration and elution methods, coliphages were detected in 69, 53, and 68% of the source, post-sedimentation, and post-sand filtration concentrates, respectively, on *E. coli* A-19 and in 96, 96, and 98% of the source, post-sedimentation, and post-sand filtration concentrates, respectively, on *E. coli* C (data not shown).

A summary of the comparative rates of recovery of

TABLE 2. Recovery of coliphages from source water by different filters

Filter (charge)	No. of samples	Recovery efficiency (%)[a] on *E. coli* C
Balston (−)	21	11 (1–41)
1MDS (+)	6	20 (1–53)
TSM (+)	6	21 (11–34)

[a] Values presented are the means, and the values in parentheses are the ranges. Values are based on the number of coliphages present in source water concentrates divided by the corresponding number of coliphages present by direct inoculation of source water.

coliphages, enteroviruses, and indicator bacteria at different stages of treatment is presented in Table 3. The treatment processes of flocculation-sedimentation and sand filtration removed ≥96% of the indicator bacteria, with the exception of an 86% removal rate for standard plate count bacteria. The same processes removed 85% of the coliphages recovered on *E. coli* A-19, 68% of the coliphages recovered on *E. coli* C, and 92% of the enteroviruses; 90% is the normal virus removal rate for flocculation-sedimentation and sand filtration (4). A comparison of yearly coliphage and enterovirus average counts in water at plant intake (compare Table 1 with Table 3) shows that coliphages outnumber enteroviruses ca. 8,115 to 1 (PFU/liter) on *E. coli* A-19 and ca. 23,425 to 1 (PFU/liter) on *E. coli* C. These data confirm that coliphage concentrations normally greatly exceeds virus concentrations. Because of the consistency with which coliphages were found, it can be stated that in this source water they are present when enteroviruses are present. Comparison of the post-sand filtration average coliphage and enteric virus levels gives a ratio of 1,235 to 1.0 (PFU/liter) on *E. coli* A-19 and 13,870 to 1.0 (PFU/liter) on *E. coli* C (Table 3). These data demonstrate the persistence and similar behavior of coliphages and enteroviruses during treatment and the excess number of coliphages to enteroviruses. Also, a Spearman correlation analysis of the data, using monthly means at each collection point, indicated that enterovirus counts were more strongly correlated with coliphage counts ($P < 0.01$, $r = 0.54$), both on *E. coli* A-19 and C, than with the bacterial indicators.

Data obtained during this study indicate that coliphages may partially satisfy the listed criteria. Coliphage and enterovirus concentrations persisted and fluctuated in a similar pattern, and coliphage concentrations in source water and throughout treatment before chlorination (which caused total removal of detectable viruses) were in excess of enterovirus concentrations. Also, water treatment processes removed coliphages and enteroviruses at a similar rate. In addition, coliphages are easily quantified by a procedure that is simple, economical, applicable to most water types, and

TABLE 3. Average rates of recovery of microorganisms from different sites within the treatment plant[a]

Site	Avg recovery rate of:						
	Coliphage (PFU/liter)		Enterovirus[b] (PFU/380 liters)	Coliforms[b] (CFU/100 ml)		Fecal streptococci[b] (CFU/100 ml)	Standard plate count[b] (CFU/1.0 ml)
	A-19	C		Total	Fecal		
Source	86	454	48	12,395	844	2,218	2,784
Post-sedimentation	27	284	18	1,646	89	228	935
Post-sand filtration	13	146	4	375	32	63	398
Finished water	<1	<1	<1	<1	<1	<1	<1

[a] The average values are based on 45 coliphage, 51 enterovirus, and 49 indicator bacteria samples.
[b] Adapted and modified from Stetler et al. (16).

requires only 8 h. Based on the host bacterium chosen, the test can be selective for a particular coliphage. Samples can also be readily tested to prevent false-positive readings. Other investigators have already established that coliphages are at least as resistant as enteroviruses to chlorination (6, 7). From these findings, it appears that coliphages have good potential as indicators of enteroviruses during water treatment. However, the potential variation in coliphage populations in surface waters could reduce their value as an indicator (3, 17). Additional data on coliphage counts at water plant intakes around the country are required before serious consideration can be given to their acceptance as a microbial indicator of finished water quality.

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